PATENT
Client-Matter No.: 66661-018
(P-IS 4373)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Biaoyang Lin	Confirmation No: 5002 Group Art Unit: 1642
Serial No.: 09/821,812	Examiner: M. Davis
Filed: March 28, 2001	
For: ANDROGEN REGULATED PROSTATE SPECIFIC NUCLEIC ACIDS	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION PURSUANT TO 37 C.F.R. §1.132

Sir:

I, Biaoyang Lin, declare as follows:

1) I am the Biaoyang Lin who is named as the sole inventor on the above-identified patent application.

2) I understand that the claims of the subject application stand rejected, in part, because it is alleged that the claimed ARP3 polypeptides and fragments lack utility.

3) The above-identified patent application indicates that ARP3 polypeptides and fragments can be useful for generating antibodies. The above-identified patent application further indicates that ARP binding agents such as antibodies can

be useful in imaging techniques, for example, for detecting secondary sites of prostate cancer metastasis. I, or those working under my direction and supervision, have performed experiments to confirm that ARP3 is differentially expressed in tissue from prostate cancer metastases as compared to non-metastatic prostate cancer tissue. These experiments are described in paragraphs 4 to 8 below.

4) A total of 14 biopsy specimens were analyzed from metastases secondary to prostate cancer or from localized, non-metastatic prostate cancers. These biopsy specimens were obtained from The University of Washington tissue bank. Specifically, biopsy specimens #1-11 were taken from various metastases secondary to prostate cancer, and biopsy specimens #12-14 were taken from localized, non-metastatic prostate cancers.

5) Real-time quantitative polymerase chain reaction (PCR) assays were performed with each biopsy specimen and the ARP3 forward and reverse primers 5'-TGACCTCATTTGAACGTGCCCTTTC-3' and 5'-CCCTTGCATAATGCTGCTTCATAAGAAC-3' to produce a 458 bp PCR fragment of the human ARP3 cDNA disclosed in the above-identified patent application. The ARP3 primers were designed such that a 9 kb intron intervened; as expected, no amplification of genomic DNA was observed.

6) Real-time PCR reactions were performed on an ABI 7700 machine (PE Biosystems) using the SYBR Green dye (Molecular Probes, Inc.) for detection. The following PCR conditions, which gave bands of the expected size and minimal primer dimers, were used: 94°C for 30 seconds; 55°C for 30 seconds and 72°C

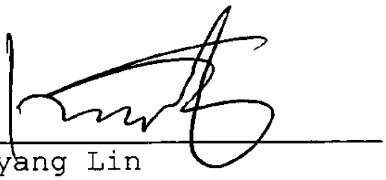
for 30 seconds for 35 cycles. To normalize RNA levels, a hypoxanthine phosphoribosyl transferase (HPRT) control fragment was also amplified. For each of the 14 biopsy specimens, the level of ARP3 RNA was quantitated relative to the level of HPRT control RNA. The threshold cycle (Ct) values of ARP3 in each sample were converted to a relative quantity using a standard curve. The results are shown in Table 1.

7) As illustrated in Figure 1 and shown in Table 1, the relative quantity of ARP3 RNA was uniformly low in the three localized, non-metastatic prostate cancer specimens (#12-14), while the relative quantity of ARP3 RNA was elevated in the majority of metastatic biopsy specimens. These data demonstrate relatively high expression of ARP3 in various different prostate cancer metastases.

8) Northern analysis was performed using a commercially prepared multiple tissue Northern blot. As shown in Figure 2, except for expression in testis and ovary and low level expression in prostate, ARP3 is not expressed at significant levels in normal tissues.

9) In sum, these results show that ARP3 is relatively highly expressed in many metastatic prostate cancers as compared to non-metastatic prostate cancer and most normal tissues. These results substantiate the asserted utility of anti-ARP3 antibodies for imaging metastatic prostate cancer.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that any such willful false statement may jeopardize the validity of the application or any patent issued thereon.

Date: Nov. 21, 2003 By: 

Biaoyang Lin

Table 1 RealTime PCR analysis comparing localized and metastasized prostate cancer.

		Relative quantity	STDEV
1	Cap mesenteric met	0.74	0.12
2	Cap met 1	1.10	0.43
3	Cap met 2	4.05	0.63
4	Cap met 3	2.10	0.33
5	Cap met 4	0.29	0.32
6	Cap met Adrenal	0.79	0.05
7	Cap Lymph Node met 3	2.92	0.68
8	Cap, liver Met	3.46	0.61
9	CaP, Lung Met	0.09	0.02
10	Met Lymph Node met 1	1.67	0.20
11	Met Lymph Node met 2	0.40	0.11
12	CaP Primary	0.43	0.06
13	CaP Primary	0.33	0.09
14	CaP Primary	0.31	0.06